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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/019,409	12/28/2001	Masahiro Iwakura	04853.0084	3873

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EXAMINER	
PROUTY, REBECCA E	

ART UNIT	PAPER NUMBER
1652	

DATE MAILED: 08/27/2003

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary

Application No.	Applicant(s)
10/019,409	Iwakura
Examiner	**Art Unit**
Rebecca Prouty	1652

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136 (a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1) ☐ Responsive to communication(s) filed on _____.

2a) ☐ This action is **FINAL.** 2b) ☒ This action is non-final.

3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11; 453 O.G. 213.

Disposition of Claims

4) ☒ Claim(s) 1-10 is/are pending in the application.

4a) Of the above, claim(s) 1-6 is/are withdrawn from consideration.

5) ☐ Claim(s) _____ is/are allowed.

6) ☒ Claim(s) 7-9 is/are rejected.

7) ☒ Claim(s) 10 is/are objected to.

8) ☒ Claims 1-10 are subject to restriction and/or election requirement.

Application Papers

9) ☐ The specification is objected to by the Examiner.

10) ☐ The drawing(s) filed on _____ is/are a) ☐ accepted or b) ☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11) ☐ The proposed drawing correction filed on _____ is: a) ☐ approved b) ☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12) ☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13) ☒ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a) ☒ All b) ☐ Some* c) ☐ None of:

1. ☐ Certified copies of the priority documents have been received.
2. ☐ Certified copies of the priority documents have been received in Application No. _____.
3. ☒ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

*See the attached detailed Office action for a list of the certified copies not received.

14) ☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

a) ☐ The translation of the foreign language provisional application has been received.

15) ☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s). 8,11
4) ☐ Interview Summary (PTO-413) Paper No(s). _____
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other:

Restriction is required under 35 U.S.C. 121 and 372.

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1.

In accordance with 37 CFR 1.499, applicant is required, in reply to this action, to elect a single invention to which the claims must be restricted.

Group I, claim(s) 1-6, drawn to a sulfur-free protein.

Group II, claim(s) 7-10, drawn to a method of producing a sulfur-free protein.

The inventions listed as Groups I and II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the only technical feature shared by groups I and II is a sulfur-free protein, however this does not constitute a special technical feature as defined by PCT Rule 13.2 as such a sulfur-free protein is found in the prior art. See for example Mazel et al.

During a telephone conversation with Nicol Fortune on 5/3/03 a provisional election was made without traverse to prosecute the invention of Group II, claims 7-10. Affirmation of this election must be made by applicant in replying to this Office action. Claims 1-6 are withdrawn from further consideration by the

examiner, 37 CFR 1.142(b), as being drawn to a non-elected invention.

Claim 10 is objected to under 37 CFR 1.75(c) as being in improper form because a multiple dependent claim must refer to other claims in the alternative only. See MPEP § 608.01(n). Claim 10 simultaneously depends on both Claims 7 and Claim 9. Accordingly, the claim has not been further treated on the merits.

Claims 7-9 are objected to as depending from non-elected Claim 1.

Claims 7-8 are objected to because of the following informalities: "by" in line 6 of part (1) of Claims 7 and 8 should be replaced with "with a"; "codons encoding sulfur-containing amino acids" lines 1-2 of part (2) of Claim 8 should be replaced with "a codon encoding a sulfur-containing amino acid"; "each" in line 2 of part (3) of Claim 8 should be deleted; "triple" in line 6 of part (2) of Claim 8 should be "double" and the word "method" should be inserted before "according to claim 8" in line 2 of Claim 9. Appropriate correction is required.

Claims 7-9 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 7 and 8 are confusing in the recitation of "L-methionine-L-alanine, L-methionine-L-serine, or L-methionine-L-proline codon" as a codon is a sequence encoding a single amino acid. It is suggested that this phrase be amended to recite "sequence encoding L-methionine-L-alanine, L-methionine-L-serine, or L-methionine-L-proline"

Claim 7, part (2) is vague and confusing in the recitation of "codons encoding sulfur-containing amino acids at another sites Ai (i = 2 to n) are substituted with codons encoding another amino acids among the 18 types of amino acid according to claim 1". Replacement with "a codon encoding methionine or cysteine at site Ai (i = 2 to n) is substituted with a codon encoding an amino acid selected from the group consisting of L-alanine, L-aspartic acid, L-glutamic acid, L-phenylalanine, L-glycine, L-histidine, L-isoleucine, L-lysine, L-leucine, L-asparagine, L-proline, L-glutamine, L-arginine, L-serine, L-threonine, L-valine, L-tyrosine, and L-tryptophan" is suggested.

Claim 8, part (4) is confusing in the recitation of "preparing a quadruple mutant, ...," as it is unclear what the ... includes.

Claim 9 is confusing in the recitation of "according to any one of (n! types) permutations and combinations of A1, A2, ...An". It is suggested that "wherein the order of stepwise

mutation sites is according to any one of (n! types) permutations and combinations of A1, A2, ...An" be replaced with "wherein each of sites A1-An are substituted in any order".

Claim 7-9 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for methods of construction the sulfur-free DHFR genes of Table 7 of the specification and a sulfur-free xylanase gene encoding SEQ ID NO:9, does not reasonably provide enablement for methods of constructing any sulfur-free enzyme having activity greater than or equal to the activity of the original protein. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with these claims.

Claims 7-9 are so broad as to encompass method of constructing any mutant sulfur-free enzyme having an activity greater than or equal to the corresponding wild-type parent. The scope of the claims is not commensurate with the enablement provided by the disclosure with regard to the extremely large number of methods of constructing any sulfur-free enzyme broadly encompassed by the claims. Since the amino acid sequence of a protein determines its structural and functional properties, predictability of which changes can be tolerated in a protein's amino acid sequence and obtain the desired activity requires a

knowledge of and guidance with regard to which amino acids in the protein's sequence, if any, are tolerant of modification and which are conserved (i.e. expectedly intolerant to modification), and detailed knowledge of the ways in which the proteins' structure relates to its function. In particular, the claims include the substitution of all methionine and cysteine residues in any enzyme. It is well known in the art that sulfur-containing residues and cysteine residues in particular are often critically important for the structure and function of many enzymes and that no other amino acid can provide for the structural stabilization inherent in the disulfide bonds present in many enzymes. However, in this case the disclosure is limited to the construction of a very few sulfur-free enzymes from only 2 specific parent genes, both of which in fact naturally were free of cysteine residues.

While recombinant and mutagenesis techniques are known, it is <u>not</u> routine in the art to screen for multiple substitutions or multiple modifications, as encompassed by the instant claims, and the positions within a protein's sequence where amino acid modifications can be made with a reasonable expectation of success in obtaining the desired activity/utility are limited in any protein and the result of such modifications is unpredictable. In addition, one skilled in the art would expect

any tolerance to modification for a given protein to diminish with each further and additional modification, e.g. multiple substitutions.

The specification does not support the broad scope of the claims which encompass methods of constructing any sulfur-free enzyme having activity greater than or equal to the activity of the original protein because the specification does **not** establish: (A) which sulfur containing proteins have may be modified without effecting activity; (B) the general tolerance of enzymes to modification and extent of such tolerance in particular with respect to the modification of cysteine residues which are very often highly important for both structure and catalytic function of many enzymes; and (C) the specification provides insufficient guidance as to which of the essentially infinite possible choices is likely to be successful.

Thus, applicants have not provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claims broadly including methods of constructing any sulfur-free enzyme having activity greater than or equal to the activity of the original protein. The scope of the claims must bear a reasonable correlation with the scope of enablement (In re

Fisher, 166 USPQ 19 24 (CCPA 1970)). Without sufficient guidance, determination of methods having the desired biological characteristics is unpredictable and the experimentation left to those skilled in the art is unnecessarily, and improperly, extensive and undue. See In re Wands 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988).

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 7-9 are rejected under 35 U.S.C. 103(a) as being unpatentable over the combined disclosures of Li et al. and Recktenwald et al., in view of Lathrop et al. and Barnett et al. (WO96/30481).

Li et al. and Recktenwald et al. teach that the oxidative lability of industrial and pharmaceutical enzymes is a well known problem in the art and that in particular the amino acid residues methionine and cysteine are highly oxidatively labile (see particularly Table 2 of Recktenwald et al. and the abstract and pages 491-493 of Li et al.). Li et al. and Recktenwald et al. further teach that the site-specific substitution of methionine

and cysteine residues has been a known strategy for the improvement of the oxidative stability of a variety of proteins of interest (see Table 2 and pages 7-8 of Recktenwald et al. and the abstract and pages 496-497 of Li et al.) and that this strategy has been successfully applied to at least subtilisin (Recktenwald et al., page 8) and α_1-antitrypsin (Li et al., page 497).

Barnett et al. teach the mutagenesis of *Bacillus* α-amylases to improve oxidative stability. Barnett teach that oxidative stability can be improved by the site-specific substitution of oxidizable amino acids such as methionine, tryptophan, tyrosine, histidine and cysteine with non-oxidizable amino acids (pages 2 and 4) and that in particular cysteine and methionine are the most oxidizable amino acids (page 4). Barnett et al. further teach the alteration of multiple amino acids within the enzyme to achieve the maximum stability (page 15). Barnett et al teach that this alteration of the enzyme can be accomplished while maintaining adequate enzymatic activity compared to the wild-type enzyme (page 3 and examples 11, 14, 16 and 17). Barnett et al. teach specific methods of constructing multiply substituted site-specific mutants which include a) constructing all possible single site mutants at several distinct methionine residues,

selecting those single mutants with the highest activity and then screening combinations of these mutations for those multiple mutants with the highest activities (see particularly Examples 3, 5-7, and 11) and b) creating double mutants by further mutating a precursor single mutant enzyme (see particularly Example 10). Construction of mutants by method a) above is includes all features of steps (2)-(4) of Claim 7 and method b) includes all features of steps (2)-(4) of Claims 8 and 9.

In view of the combined disclosures of Li et al. and Recktenwald et al. the ordinary skilled artisan would have found it obvious to use site specific mutagenesis to replace all the methionine and cysteine residues of any enzyme which is used in oxidative conditions with more oxidatively stable amino acids in order to improve the oxidative stability of the protein. Furthermore, the skilled artisan would have found it obvious to use either of the methods of making multiple mutants taught by Barnett et al. for making multiple mutants of *Bacillus* α-amylase, as Barnett et al. showed that these methods resulted in oxidatively stable enzymes with activity at least equal to that of the wild type enzyme. As ATG (which encodes methionine) is the initiation codon of virtually all known genes, one of ordinary skill in the would have recognized that replacing all

methionine residues would require a means for altering the N-terminal methionine while still allowing for translation initiation.

Lathrop et al. teach a method for mutating a recombinant protein produced in *E. coli* such that the methionine residue encoded by the initiation codon will be cleaved by the methionine aminopeptidase activity of the host cell. Lathrop et al. teach altering the penultimate amino acid residue codon (i.e., the residue immediately following the initiation codon) such that the first two amino acids encode a sequence which is a good substrate for *E. coli* methionine aminopeptidase and screening for a mutant enzyme that lacks an amino-terminal methionine residue but maintains activity.

Therefore, it would have been further obvious to one of skill in the art to combine the method of Lathrop et al. with the mutagenesis methods discussed above to construct an enzyme lacking any methionine residues. One of skill in the art would have been motivated to do so by the disclosures of Li et al. and Recktenwald et al. that the oxidative lability of industrial and pharmaceutical enzymes is a well known problem in the art which can be overcome by replacement of oxidatively labile amino acid residues.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Rebecca Prouty, Ph.D. whose telephone number is (703) 308-4000. The examiner can normally be reached on Monday-Friday from 8:30 to 4:30.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ponnathapu Achutamurthy, can be reached at (703) 308-3804. The fax phone number for this Group is (703) 308-4242.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Group receptionist whose telephone number is (703) 308-0196.

Rebecca Prouty
Primary Examiner
Art Unit 1652